ANTICANCER RESEARCH *25*: 4595-4598 (2005)

Interleukin-4 in Patients with Prostate Cancer

TAKESHI UEDA[1] MARIANNE D. SADAR[2], HIROYOSHI SUZUKI[1], KOICHIRO AKAKURA[3], SHINICHI SAKAMOTO[1], MASAKI SHIMBO[1], TAKAHITO SUYAMA[1], TAKASHI IMAMOTO[1], AKIRA KOMIYA[1], NAYA YUKIO[1] and TOMOHIKO ICHIKAWA[1]

[1]Department of Urology, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba-shi, Chiba 260-8670, Japan;
[2]Genome Sciences Centre, British Columbia Cancer Agency, Suite 100, 570 West 7th Ave, Vancouver, British Columbia V5Z 4S6, Canada;
[3]Department of Urology, Tokyo Kousei Nenkin Hospital, Shinjuku-ku, 5-1 Tsukudo-cho, Shinjuku-ku, Tokyo 162-8543, Japan

Abstract. *Background: Ligand-independent activation of the androgen receptor (AR) by cytokines has been implicated in the progression of androgen-independent prostate cancer (PCa). To determine the potential effects of elevated levels of interleukin-4 (IL-4) in patients with PCa, six different cytokines were examined for their ability to activate the AR. Materials and Methods: LNCaP cells were transiently transfected with prostate-specific antigen (PSA) (–630/+12)-luciferase and treated with R1881, six kinds of cytokines including IL-4, or vehicle. Transactivation assays were also performed in LNCaP cells co-transfected with the 5xGal4UAS-TATA-luciferase and AR-(1-558)-Gal4DBD prior to incubation with R1881, IL-4, IL-6, or vehicle. Seventy-two patients with pre-treatment PCa, 17 patients with hormone-refractory metastatic PCa receiving androgen ablation therapy, 20 patients with benign prostatic hypertrophy and 10 healthy male volunteers were enrolled in this retrospective study. The concentration of serum IL-4 was measured by chemiluminescence enzyme immunoassay. Results: IL-4 induced androgen-response element-driven reporters and activated the AR N-terminal domain (NTD) in a ligand-independent manner in transiently transfected LNCaP cells. Levels of IL-4 in the serum were significantly elevated in patients with hormone-refractory PCa as compared to the levels in pre-treatment PCa. Conclusion: IL-4 serum levels were demonstrated to be increased in hormone-refractory PCa and IL-4 was shown to enhance PSA reporter gene activity by the activation of AR NTD in human LNCaP cells. These results suggest that the AR can be activated by cytokines, and that this mechanism may play an important role in the transition from androgen-dependent to androgen-independent PCa after patients receive androgen ablation therapy.*

Correspondence to: Takeshi Ueda, MD, Ph.D., Division of Urology, Chiba Cancer Center, 666-2 Nitona-cho, Chuo-ku, Chiba-shi, Chiba 260-8717, Japan. Tel: +81-43-264-5431, Fax:+81-43-265-9515, e-mail: ueda3713@yahoo.co.jp

Key Words: Prostate cancer, interleukin-4, interleukin-6, androgen receptor.

Interleukin-4 (IL-4) is a pleiotropic type I cytokine that plays a central role in regulating inflammatory and cell-mediated immune responses (1). In addition to its effect on immune cells, IL-4 has a variety of functions including effects on hematopoietic tissues, tissue adhesion and inflammation (1). IL-4 exerts its function through activation of the IL-4 receptor, designated IL-4Rα, by tyrosine phosphorylation (2). IL-4Rα activation results in tyrosine phosphorylation of multiple receptor-associated kinases including the Janus-family (Jak) tyrosine kinases (Jak1, Jak2, and Jak3) (3), insulin receptor substrate (IRS-1/2) proteins (4), Shc (5) and signal transducers and activators of transcription (Stat6) (6) for the initiation of signal transduction. Recent studies have demonstrated that IL-4 levels are significantly elevated in hormone-refractory prostate cancer (PCa) compared with values in hormone-sensitive PCa, and that IL-4 levels are directly correlated with elevated levels of serum prostate-specific antigen (PSA) (7). These observations suggest a potential role of IL-4 in the development and progression of androgen-independent PCa. In this study, IL-4 serum levels in patients with PCa and the activation of the androgen receptor (AR) N-terminus by IL-4 in human PCa cells were examined.

Materials and Methods

Cells and transfection. LNCaP human PCa cells were maintained as described previously (8-11). All cytokines were obtained from R&D Systems (Minneapolis, MN, USA). The human AR cDNA was a gift from Dr. A.O. Brinkmann (Erasmus University, The Netherlands).

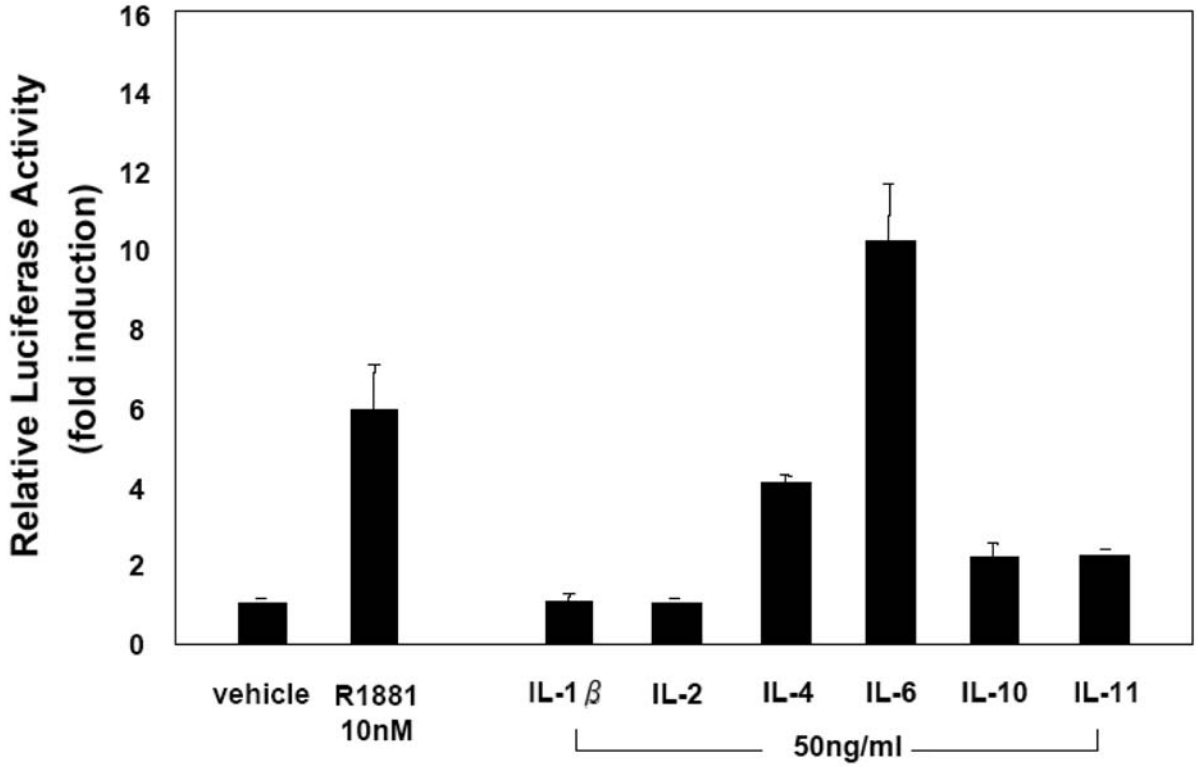

Figure 1. *Effects of R1881 and cytokines on PSA-luciferase activity in LNCaP cells. LNCaP cells were transiently transfected with PSA (–630/+12)-luciferase (1 μg/well) for 24 h and then incubated with R1881 (10 nM), cytokines (50 ng/ml), or vehicle for an additional 48 h under serum-free conditions. The error bars represent the mean±S.E. of three independent experiments.*

PSA (–630/+12)-luciferase, AR-(1-558)-Gal4 DBD, Gal4 DBD and the p5xGal4UAS-TATA-luciferase were described previously (8-10). Transfection and the luciferase assay were performed according to previously published methods (8-11). The results are presented as fold induction, which is the relative luciferase activity of the treated cells divided by that of the control cells. All transfection experiments were carried out in triplicate wells and repeated three to five times using at least two sets of plasmids that were prepared separately.

Human blood samples. Seventy-two patients (average age ± standard deviation of 70±7.0 years) with pre-treatment PCa and 17 patients (69±7.1 years) with hormone-refractory metastatic PCa receiving androgen ablation therapy at Chiba University Hospital, Japan, between 1994 and 2002, were enrolled in this retrospective study. In the same time period, 20 patients (73±7.3 years) in whom PCa was not detected (non-PCa) were enrolled. Ten healthy male volunteers (30±4.6 years) were also entered into this study. Histological diagnoses of PCa and non-PCa were confirmed by needle biopsy. All blood samples were obtained with written informed consent. The blood samples were immediately clotted and the sera frozen and stored at –20˚C until analysis. The concentration of serum IL-4 was measured by chemiluminescence enzyme immunoassay (SRL, Tokyo, Japan). The serum PSA level was determined at least once every two months using the Tandem-R kit (Hybritech, San Diego, CA, USA). Statistical analysis was performed by Chi-square test. $P<0.05$ was considered to be significant.

Results

Androgen-independent induction of the PSA promoter gene by cytokines. Progression of PCa to androgen independence is monitored in patients by elevated levels of serum PSA, an androgen-regulated gene. IL-6 has been suggested to play an important role in the progression of PCa to androgen independence by a mechanism involving ligand-independent activation of AR through its N-terminus to increase transcription of androgen-regulated genes (9, 10). Here, five other cytokines were screen for their ability to activate AR by measurement of PSA expression in the LNCaP human prostate cancer cell line. LNCaP cells were transiently transfected with the PSA (–630/+12)-luciferase reporter plasmid. R1881 (10 nM) and IL-6 (50 ng/ml) were used as positive controls. Treatment of transiently-transfected LNCaP cells to IL-4 (50 ng/ml) resulted in a 4-fold increase in PSA-reporter activity relative to the control (Figure 1). IL-1β, IL-2, IL-10 and IL-11 showed negligible effects on PSA-luciferase activities. These results indicate that IL-4 causes androgen-independent increases in PSA-luciferase activity in LNCaP cells.

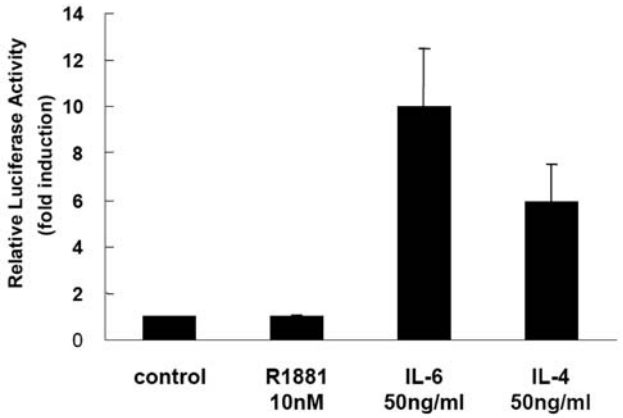

Figure 2. *Effect of IL-4 on the activity of the human AR NTD. Transactivation assays were performed in LNCaP cells co-transfected with 5xGal4UAS-TATA-luciferase (1 μg/well) and AR-(1-558)-Gal4DBD (50 ng/well) or Gal4DBD for 24 h prior to incubation with R1881 (10nM), IL-6 (50 ng/ml), IL-6 (50 ng/ml), or vehicle for an additional 24 h. The total amount of plasmid DNA transfected was normalized to 3 μg/well by addition of the empty vector. The error bars represent the mean±S.E. of three independent experiments and are normalized to respectively treated Gal4DBD (vector alone) values.*

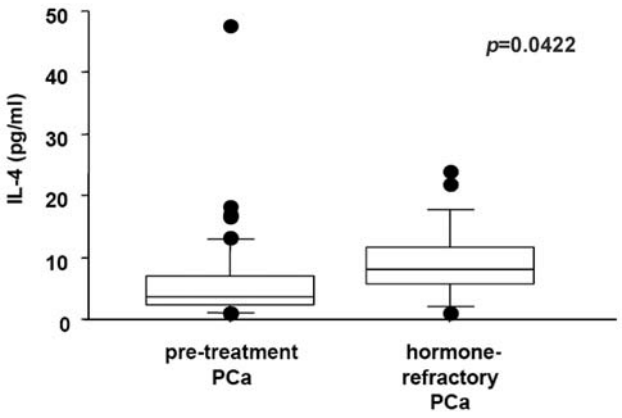

Figure 3. *Comparison of serum IL-4 value between pre-treatment (n=72) and hormone-refractory PCa (n=17). The error bars represent the mean±S.D.*

IL-4 activates the human AR NTD. Ligand-independent activation of AR has been shown to involve the N-terminal domain (NTD) for its activation by IL-6 (9, 10). Here, it can be seen that IL-4 induces the PSA promoter that contains two, well-characterized androgen response elements. This suggests that IL-4 may also activate the AR. To test this, LNCaP cells were co-transfected with the expression vector encoding AR-(1-558)-Gal4DBD and a reporter gene containing the Gal4-binding site. R1881 was used as a negative control since it does not bind to this region of the AR. IL-6 has previously been shown to activate a similar system (9) and was included here as a positive control. Exposure of cells to IL-4 resulted in a 6-fold increase in Gal4-luciferase activity (Figure 2). These data suggest that the AR NTD is a target of the IL-4 signaling pathway.

Elevated IL-4 in patients with hormone-refractory PCa. AR activation by IL-4 in the absence of androgens suggests a possible mechanism for progression of PCa to androgen independence. To determine the disease relevance of these data, IL-4 levels were examined in hormone-refractory PCa patients receiving androgen ablation therapy. The distribution of clinical stage and histological grade in PCa patients enrolled in this study is summarized in Table I. IL-4 values were significantly elevated in patients with hormone-refractory PCa compared to the pre-treatment PCa ($p<0.0422$, Figure 3). IL-4 serum levels did not significantly differ between the healthy volunteers, the patients with benign prostatic enlargement and pre-treatment PCa patients. Furthermore, IL-4 levels did not significantly differ in pre-treatment patients with regard to clinical stage, histological grade and Gleason score of the tumor. In PCa patients before treatment and hormone-refractory status, IL-4 serum levels did not correlate with PSA serum levels.

Discussion

The AR can be activated in the absence of its cognate ligand by growth factors, modulation of protein kinase pathways and by IL-6 (8-12). Ligand-independent activation by IL-6 of endogenous AR in human PCa cells by targeting the AR NTD to enhance AF-1 activity has been reported (9, 10). The events involved in ligand-independent AR activation by alternative pathways are unknown, but have been suggested to involve phosphorylation of the AR NTD itself, or a receptor-associated protein that interacts with this AR domain. Ligand-independent activation of the AR has been suggested to be a possible mechanism underlying androgen-independent prostate cancer (8-11, 13).

IL-4 serum levels are significantly elevated in hormone-refractory PCa compared with values in hormone-sensitive PCa, and these IL-4 levels directly correlated with elevated PSA (7). PSA expression is tightly regulated by androgen through the action of AR binding to AREs on the PSA promoter. In this study, it was demonstrated that serum levels of IL-4 increased in hormone-refractory PCa, and that IL-4 induced PSA reporter activity through the activation of AR NTD in LNCaP PCa cells. These studies suggest that IL-4 induction of PSA gene expression may act through an AR-dependent pathway. However, recently, Lee *et al*. have shown the role of signaling pathways such as PI3K-Akt, but not MAPK, in the induction of PSA gene expression by IL-4 (14).

Table I. *PSA and IL-4 serum levels in patients with and without prostate cancer.*

	Patients (n)	PSA Mean±S.D. (range; ng/ml)	IL-4 Mean±S.D. (range; pg/ml)
Healthy volunteer males	10	0.4±0.3 (0.3-1.0)	3.0±4.1 (0.4-15)
Non-PCa	20	6.4±7.3 (0.3-30)	5.2±5.2 (0.9-20)
PCa	72	210±883 (0.8-6991)	5.8±6.7 (1.1-47.4)
HRPCa	17	1001±1291 (32-4400)	9.2±5.9 (1.0-24.0)
Clinical stage			
T1cN0M0/T2N0M0	29	10.5±7.5 (0.8-32.4)	5.7±6.0 (1.0-18.1)
T3N0M0	23	48.91±100 (1.0-431)	4.8±4.4 (1.0-13.4)
TxNxM1	20	686±1603 (4.6-6991)	7.7±11.1 (1.1-47.7)
Histological grade			
Well	14	42±112 ((1.0-431)	6.6±4.9 (1.0-16.8)
Moderate	40	57±175 (0.8-1092)	5.1±4.7 (1.0-18.1)
Poor	18	682±1692 (0.8-6991)	6.1±10.6 (1.1-47.7)

HRPCa: hormone-refractory prostate cancer

In summary, we demonstrated that IL-4 serum levels increase in hormone-refractory PCa and that this cytokine enhanced PSA reporter gene activity by the activation of AR NTD in human LNCaP cells. These results suggest that the AR can be activated by cytokines, and this mechanism may play an important role in the transition from androgen-dependent to androgen-independent prostate cancer after patients have received androgen ablation therapy.

Acknowledgements

This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, and Culture (14571483, 16591582, 16689026, 17591663), Japan and Health Canada for the Vancouver Centre of Excellence for Prostate Cancer Research (MDS).

References

1 Nelms K, Keegan AD, Zamorano J, Ryan JJ and Paul WE: The IL-4 receptor: signaling mechanisms and biologic functions. Annu Rev Immunol *17*: 701-738, 1999.

2 Smerz-Bertling C and Duschl A: Both interleukin 4 and interleukin 13 induce tyrosine phosphorylation of the 140-kDa subunit of the interleukin 4 receptor. J Biol Chem *270*: 966-970, 1995.

3 Johnston JA, Kawamura M, Kirken RA, Chen YQ, Blake TB, Shibuya K, Ortaldo JR, McVicar DW and O'Shea JJ: Phosphorylation and activation of the Jak-3 Janus kinase in response to interleukin-2. Nature *370*: 151-153, 1994.

4 Wang LM, Myers MG Jr, Sun XJ, Aaronson SA, White M and Pierce JH: IRS-1: essential for insulin- and IL-4-stimulated mitogenesis in hematopoietic cells. Science *261*: 1591-1594, 1993.

5 Zhou MM, Huang B, Olejniczak ET, Meadows RP, Shuker SB, Miyazaki M, Trub T, Shoelson SE and Fesik SW: Structural basis for IL-4 receptor phosphopeptide recognition by the IRS-1 PTB domain. Nat Struct Biol *3*: 388-393, 1996.

6 Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, Chu C, Quelle FW, Nosaka T, Vignali DA, Doherty PC, Grosveld G, Paul WE and Ihle JN: Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. Nature *380*: 630-633, 1996.

7 Wise GJ, Marella VK, Talluri G and Shirazian D: Cytokine variations in patients with hormone treated prostate cancer. J Urol *164*: 722-725, 2000.

8 Sadar MD: Androgen-independent induction of prostate-specific antigen gene expression *via* cross-talk between the androgen receptor and protein kinase A signal transduction pathways. J Biol Chem *274*: 7777-7783, 1999.

9 Ueda T, Bruchovsky N and Sadar MD: Activation of the androgen receptor N-terminal domain by interleukin-6 *via* MAPK and STAT3 signal transduction pathways. J Biol Chem *277*: 7076-7085, 2002.

10 Ueda T, Mawji NR, Bruchovsky N and Sadar MD: Ligand-independent activation of the androgen receptor by interleukin-6 and the role of steroid receptor coactivator-1 in prostate cancer cells. J Biol Chem *277*: 38087-38094, 2002.

11 Blaszczyk N, Masri BA, Mawji NR, Ueda T, McAlinden G, Duncan CP, Bruchovsky N, Schweikert HU, Schnabel D, Jones EC and Sadar MD: Osteoblast-derived factors induce androgen-independent proliferation and expression of prostate-specific antigen in human prostate cancer cells. Clin Cancer Res *10*: 1860-1869, 2004.

12 Culig Z, Hobisch A, Cronauer MV, Radmayr C, Trapman J, Hittmair A, Bartsch G and Klocker H: Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. Cancer Res *54*: 5474-5478, 1994.

13 Sadar MD, Hussain M and Bruchovsky N: Prostate cancer: molecular biology of early progression to androgen independence. Endocr Relat Cancer *6*: 487-502, 1999.

14 Lee SO, Lou W, Hou M, Onate SA and Gao AC: Interleukin-4 enhances prostate-specific antigen expression by activation of the androgen receptor and Akt pathway. Oncogene *22*: 7981-7988, 2003.

Received January 20, 2005
Accepted July 11, 2005